Hasanuddin J. Anim. Sci. Vol. 4, No. 2: 125-136
pISSN 2621-9182 eISSN 2621-9190
DOI:10.20956/hajas.v4i2.21798

Level of DNA Similarity the Horned and Polled Bali Cattle Using Microsatellite Approach

Zulkharnaim[a*], Sudirman Baco[a], LellahRahim[a], Muhammad Yusuf[a]

[a]Departementof Animal Production, Faculty of Animal Science, Hasanuddin University, Jl. PerintisKemerdekaan Km.10, Makassar, Indonesia 90245

*Corresponding author: E-mail: zulkharnaim@unhas.ac.id

ARTICLE INFO

Article history:
Submission: July 19, 2022
Accepted: January 22, 2023
Published: February 16, 2023

ABSTRACT

The phenomenon of polled on Bali cattle should have a scientific explanation that explains the validity of the breed. Whether Bali polled cattle still have a genetic relationship with Bali horns cattle or are the results of crosses with other cattle breeds remain unclear. This study used microsatellite markers to identify some of the genetic diversity and relationship between Bali polled and Bali horn in cattle. Samples of 40 polled and 189 horned Bali cattle aged 2.5-3.5 years old were used in this study. Research data analysis includes: genotype and allele frequencies, Hardy Weinberg Equilibrium analyze, observed and expected heterozygosity values, and polymorphic informative content. Results showed that Bali polled cattle have similarity genetic characteristics with Bali horn cattle based on HEL9, INRA035, ILSTS045, and HEL13 microsatellites. HEL9, INRA035, ILSTS045, and HEL13 microsatellite can be a genetic marker for Bali polled cattle.
Keywords: Bali polled cattle, genetic similarity, microsatellite, specifics traits, genetic marker

INTRODUCTION

Bali Polled cattle are cattle whose horns do not grow naturally. The phenomenon of not growing horns in Bali cattle is still being investigated whether it is a result of a genetic mutation or the result of crossing with other cattle breeds. Bali cattle is an Indonesian domestic livestock originating from domesticated wild Banteng (*Bibos banteng)*, *Bos javanicus*, *Bos sondaicus* [1] have a distinctive genetic characteristic. This condition makes Bali cattle different from other cattle breeds in the world. A variant of Bali polled cattle that had been identified earlier might be naturally hornless progenies of homozygous generations [2].

Before cattle were domesticated, horn function is essential for the survival of wild species [3]. This function is mainly as an instrument in defending against other animal threats [4]. Horns have a function related to the behavior patterns of cattle, the presence of horns is related to the quality and quantity of social interactions and social relationships in a cattle population. The phenomenon of not growing horns in cows is categorized under two

conditions, 1) it is said to polled if the horns do not grow naturally and 2) the condition of the scurs, which is the non-growth of horns caused by failure to join the core of the hornbones with the skull [5], [3].

Figure 1. Bali polled cattle at Faculty of Animal Science Laboratory, Hasanuddin University

Development of beef cattle in the world today lead to the development of cattle without horns (polled), due to several advantages, especially in the field of maintenance management excellence. Most of beef cattle and dairy cattle in the world have implemented a maintenance model in pasture, so that the presence of horns is considered to have a relatively small value, and even tends to give a considerable economic loss impact due to a higher risk of injury (infection, damage carcass, etc.) [3]. Several countries have implemented animal welfare related to dehorning, so that breeding polled cattle is more profitable. In Simmental cattle, have been many efforts to produce pure nation polled Simmental through a selection of traditional phenotypic. This effort has taken 25 years [6].

Bali cattle as one of the local cattle in Indonesia basically have horns, both male and female. Bali cattle males have horns of different sizes with females; males generally are 20 to 25 centimeters, while the females are shorter than male-owned horn [1]. The phenomenon of polled on Bali cattle should have a scientific explanation that explains the validity of the breed. Whether Bali polled cattle still have a genetic relationship with horns cattle or are the results of crosses with other cattle breeds. This study used microsatellites markers to identify the genetic diversity and relationship between Bali polled andBali horns cattle.

MATERIALS AND METHODS

The research was conducted at the integrated biotechnology laboratory and Maiwa Breeding Center, Faculty of Animal Husbandry, Hasanuddin University in November 2016-November 2017. The number of Bali cattle samples was 40 Bali polled and 189 Bali horns. The age of the study sample was 2.5-3.5 years.

Amplification of Microsatellite Markers

Genetic material comes from DNA samples taken from whole blood. The DNA was isolated and purified using the Gene jet Genomic DNA Extraction (Thermo Scientific) DNA extraction kit following the extraction protocol provided.

The composition of the PCR reaction was conditioned at 25µl reaction volume consisting of 100 ng DNA, 025 mM each primer, 150uM dNTP, 2.5 mM Mg^{2+}, 0.5 U Taq DNA polymerase and 1x buffer. The PCR machine conditions began with initial denaturation at 94° C for 2 minutes, followed by the next 35 cycles each with denaturation of 94° C for 45 seconds, with the annealing temperature for each microsatellite presented in Table 1, then continued with extension: 72^{o}C for 60 seconds, ending with one final extension cycle at 72^{o}C for 5 minutes using a PCR machine (Senso Quest, Germany). PCR product analysis and detection of microsatellite alleles were carried out by electrophoresis on 10% polyacrylamide gel and staining with silver [7].

Table 1. Sequence and Primer Size of Microsatellites

Name of Microsatellite	Primer Sequence (5'-3')	*Anneling* (°C)	Size (bp)	Reference
HEL9	F: CCCATTCAGTCTTCAGAGGT R: CACATCCATGTTCTCACCAC	55	143-165	[8]
INRA035	F: ATCCTTTGCAGCCTCCACATTG R: TTGTGCTTTATGACACTATCCG	55	120	[9]
ILSTS045	F: TTCTGGCAAACTATTCCACC R: CATGAAAGACACAGATGACC	57	144-198	[10]
HEL13	F: TAAGGACTTGAGATAAGGAG R: CCATCTACCTCCATCTTAAC	55	174-204	[11]
ILSTS017	F: GTCCCTAAAATCGAAATGCC R: GCATCTCTATAACCTGTTCC	60	105-125	[10]

F = Forward, R = Reverse

Data Analyses

Genotypic Study

The value of the genotype, allele frequencies, and Hardy Weinberg Equilibrium (HWE) value were calculated by the following formula: Genotype and Allele Frequencies [12].

$$x_{ii} = {}^{n_{ii}}/_{N}$$

$$x_i = \left(2n_{ii} + \sum n_{ii}\right)/2n$$

Where x_{ii} = frequency of genotype ii; x_i = frequency of alel*i*; n_{ii} = total individuals with genotype *ii*; n_{ij} = total individuals with genotype *ij*; and *N* = population size.

Hardy Weinberg Equlibrium (HWE) [13].

$$x^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

Where χ^2 = chi-square; *O* = total of observations genotype to-*i*; *E* = total of genotype to expectations to-*i*.

The observed and expected heterozygosity values were calculated as follow [14]:

$$H_e = 1 - \sum_{i=1}^{n} X_i^2$$

$$H_o = \sum_{i \neq j} \frac{N_{1ij}}{N}$$

Where H_e is the expected heterozygosity; H_o is the observed heterozygosity; X_i is the frequency of i^{th} allele; N_{ij} is the number of heterozygote sample; and *N* is the number ofobservation.

The polymorphic informative content was calculated as follows [14]:

$$PIC = 1 - \sum_{i=1}^{n} X_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} X_i^2 X_j^2$$

Where PIC is polymorphic informative content; X_i is the frequency of i^{th} allele; and X_j is the frequency of j^{th} allele.

RESULTS AND DISCUSSIONS

The results of this study are shown in Figure 2 to Figure 6 and Table 2 and Table 3. All five microsatellites using in this study showed polymorphism for evaluating genetic variation within breed and exploring genetic differences between breeds (Figure 2-6). There two genotypes are found at microsatellite HEL9 in Bali polled cattle (AA and BB genotype), but BB genotype was found only in Bali polled cattle (Figure 2and Table 2). The results HEL9 amplification using microsatellite markers showed the highest frequency of the AA genotype (homozygous). Bali Polled cattle are 0.909 while horns are 1.000 (monomorphic).The highest genotype frequency in INRA035 microsatellite is found in AB genotype where Bali polled cattle are 1.000 while Bali horns cattle are 0.944 (Figure 3 and Table 2).Different results are found in the ILSTS017 microsatellite, where the GJ genotype (frequency 0.326) is only found in Bali horns cattle (Table 2).

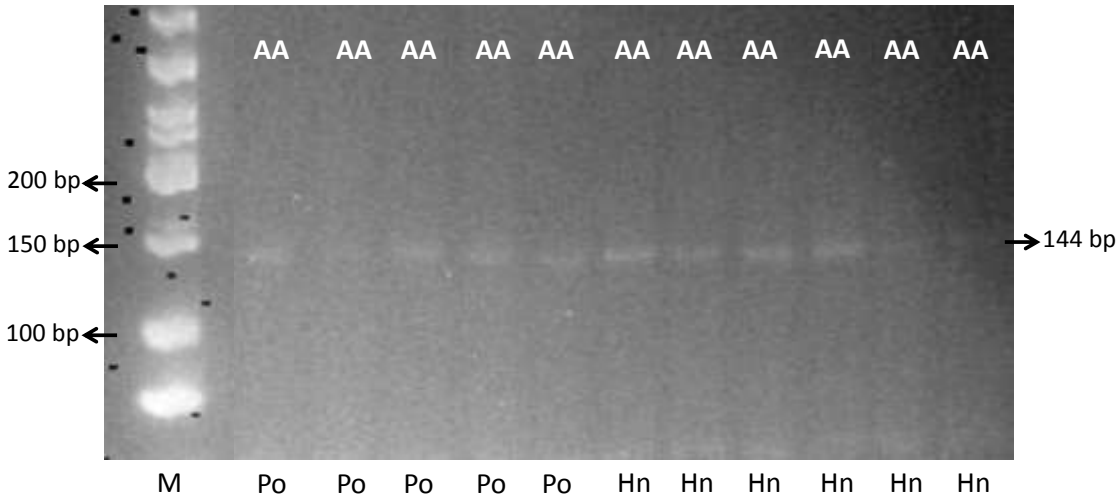

Figure 2. Microsatellite HEL9 Amplification Results; M=Marker; Po=Polled, Hn=Horned; AA=AA Genotipe (144 bp)

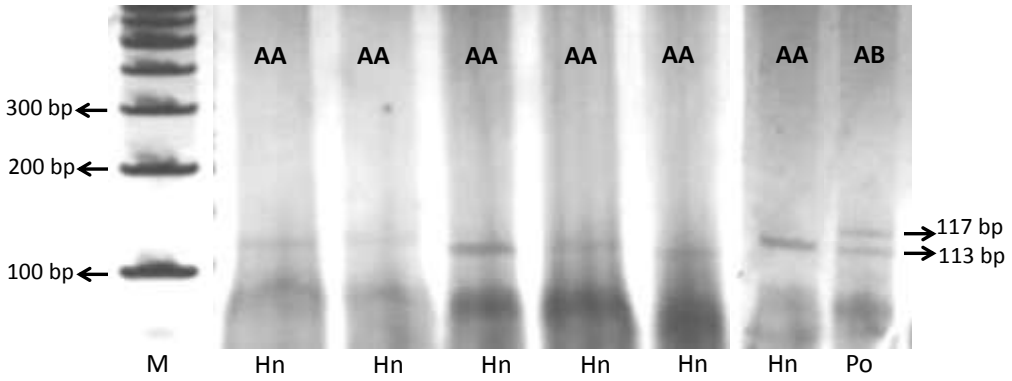

Figure 3. Microsatellite INRA035 Amplification Results; M=Marker; Po=Polled, Hn=Horned; AA=AA Genotipe (113 bp); AB=AB Genotipe (113 and 117 bp)

HEL9 and INRA035 microsatellites have been used to identify similarity of Banteng and Bali cattle [20]. In Bali polled cattle there are some alleles that have low frequency. At HEL9 locus in Bali horns cattle, highest frequency is found at A allele (1.000) be categorized as monomorphic. At A allele in Bali polled cattle, cannot be categorized as monomorphic, because the frequency is only 0.909 or 90.9%. If the number of allele is ≥0.95, then this allele can be categorize as monomorphic and specific [13], [20]. The high frequency in A allele at HEL9 locus in both of Bali polled and Bali horns cattle indicates that are identical. The identical A allele at HEL9 locus in Bali cattle are found in other research in Bali cattle [19],[24],[25].

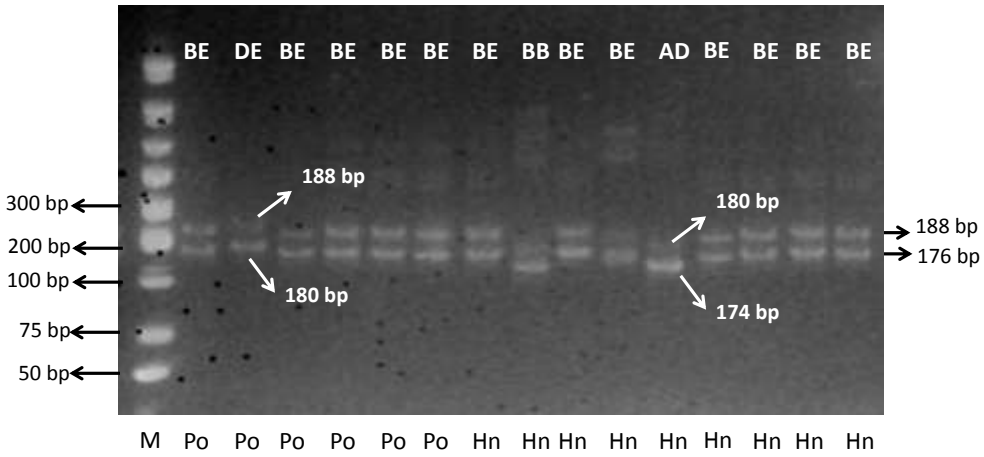

Figure 4. Microsatellite ILSTS045 Amplification Results; M=Marker; Po=Polled, Hn=Horned; BE=BE Genotipe (176&188 bp); AD=AD Genotipe (174 and 180 bp); DE=DE Genotipe (180 and 188 bp)

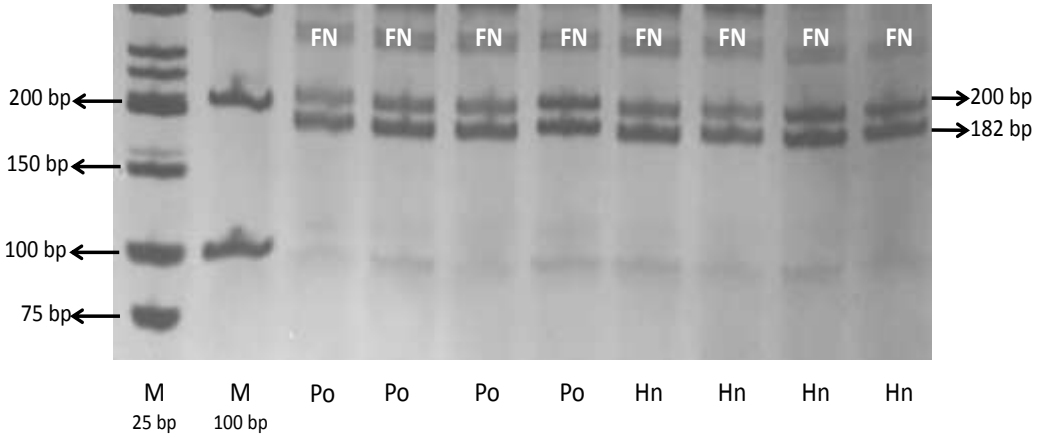

Figure 5. Microsatellite HEL13 Amplification Results; M=Marker; Po=Polled, Hn=Horned; FN=FN Genotipe (182 and 200 bp)

On the other hand, AB genotype at INRA035 locus is identified that the highest genotype frequency in Bali polled and Bali horns cattle. The AB genotype is heterozygous and same results were found in other research in Bali cattle [20],[24],[25]. Two alleles at INRA035 locus, A and B alleles be categorized as polymorphic in Bali cattle. The breed of cattle that also has C allele at INRA035 locus is Madura and Ongole [20]. The A and B alleles at INRA035 locus be categorized specific alleles and that are identical in Bali cattle.

The similar condition is found in the ILSTS045 and HEL13 microsatellites where the highest frequency is found in the BE and FN genotypes (Figure 4, 5, and Table 2). There are four alleles in ILSTS045 microsatellite, A-B-D-E alleles were found in Bali polled and Bali horns cattle. Both of Bali polled and Bali horns cattle, the high frequency allele is E and then B (Table 2). At genotype frequency, BE genotype has high frequency. There is similarity in Bali polled and Bali

horns cattle about dominant (high frequency) allele and genotype. That is indicated that Bali polled and horns cattle has the same identical allele and genotype in ILSTS045 microsatellite.

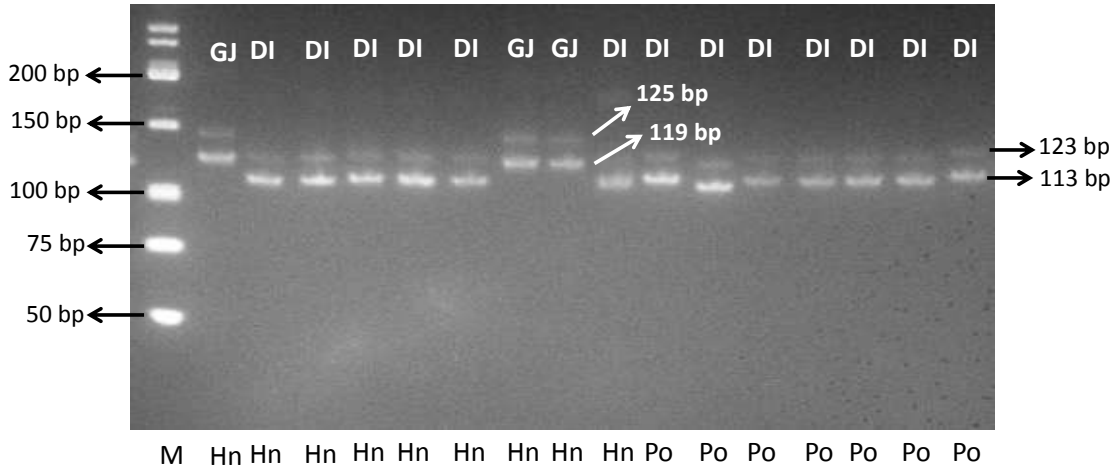

Figure 6. Microsatellite ILSTS 017 Amplification Results; M=Marker; Po=Polled, Hn=Horned; GJ=GJ Genotipe (119 and 125 bp); DI=DI Genotipe (113 and 123 bp)

Identification of DNA similarity Bali polled cattle conducted to determine the genetic relationships with Bali horns cattle (Table 2 and 3). At HEL13 there are two alleles and two genotypes. FN genotype in Bali polled cattle ispolymorphic because the frequency is ≤0.95. Whereas, FN genotype in Bali horns cattle is monomorphic (0.989). The HEL9, INRA035 and HEL13 microsatellites are recommendation from FAO for the analysis of genetic distances within each domestic animal species [26]. There is similar condition is found in Bali polled and Bali horns cattle, that FN genotype is dominant genotype. These conditions describe that polled and horned Bali cattle are identical.

Bali polled and Bali horns cattle show a close genetic relationship seen from the high frequency of the same genotypes and alleles (HEL9, INRA035, ILSTS045 and HEL013). ILSTS017 microsatellite in polled cattle was monomorphic. Dominant allel in four microsatellites have similarity. Dominant allele in polled cattle were found in Bali horns cattle. The use of several molecular markers (DNA Markers) such as microsatellites has now been able to identify differences between one livestock breed and another, especially in relation to efforts to preserve and identify the similarity of these breeds. Microsatellites are the best and important choice for the characterization of cattle lines or populations [15], [16].

The genetic markers are often used to answer questions related to genetic diversity and genetic relationships among populations of cattle [16], [17], [18]. Microsatellite loci used in this study are microsatellites that are often used to identify genetic diversity, characterization and genetic relationships in local cattle [15], [20]. The results of amplification of HEL9 microsatellites (genotype AA) and INRA035 (genotype AB) were not only found in Bali cattle but also in Banteng (*Bibos banteng*) as the domestication parents of Bali cattle [20].

Table 2. Genotype and Allele Frequencies of Bali Polled and Bali Horns Cattle Microsatellites

Microsatellite Marker	Allele Frequencies		Genotype Frequencies	
HEL9 (Polled)	A	(0.909)	AA	(0.909)
	B	(0.091)	BB	(0.091)
HEL9 (Horned)	A	(1.000)	AA	(1.000)
	B	(0.000)	BB	(0.000)
INRA035 (Polled)	A	(0.500)	AA	(0.000)
	B	(0.500)	AB	(1.000)
			BB	(0.000)
INRA035 (Horned)	A	(0.494)	AA	(0.022)
	B	(0.506)	AB	(0.944)
			BB	(0.034)
ILSTS045 (Polled)	A	(0.091)	AD	(0.182)
	B	(0.364)	BE	(0.727)
	D	(0.136)	DE	(0.091)
	E	(0.409)		
ILSTS045 (Horned)	A	(0.084)	AD	(0.169)
	B	(0.360)	BE	(0.719)
	D	(0.140)	DE	(0.112)
	E	(0.416)		
HEL 13 (Polled)	F	(0.545)	FF	(0.091)
	N	(0.455)	FN	(0.909)
HEL 13 (Horned)	F	(0.506)	FF	(0.011)
	N	(0.494)	FN	(0.989)
ILSTS 017 (Polled)	D	(0.500)	DI	(1.000)
	G	(0.000)	GJ	(0.000)
	I	(0.500)		
	J	(0.000)		
ILSTS 017 (Horned)	D	(0.337)	DI	(0.674)
	G	(0.163)	GJ	(0.326)
	I	(0.337)		
	J	(0.163)		

FAO has specified a minimum of four distinct alleles per locus for proficient judgment of genetic differences between breeds [21]. In this study of the five microsatellites is used, whereas four microsatellites have same at dominant alleles condition (frequency) in polled and horned Bali cattle. The result indicates that the alleles of polled and horned Bali cattle are identical. The heterozygosity value of both observed heterozygosity (H_o) and expected heterozygosity (H_e) is an accurate way to measure genetic variation.

Table 3. Information of Analyzed Markers in The Polled and Horned Bali Cattle

Microsatellite Marker	Observed Size Range (bp)	n_A	FNA	H_o	H_e	HWE	PIC
HEL9:							
1. Polled	143-165	2	0.909 (*A*)	0.000	0.165	0.333	0.152
2. Horned			1.000 (*A*)	0.000	0.000	1.333	0.000
INRA035:							
1. Polled	120	2	0.500 (*B*)	0.500	0.500	4.167	0.000
2. Horned			0.506 (*B*)	0.944	0.500	1.500	0.180
ILSTS045:							
1. Polled	144-198	4	0.409 (*E*)	1.000	0.674	0.429	1.500
2. Horned			0.416 (*E*)	1.000	0.671	0.441	1.500
HEL13:							
1. Polled	174-204	2	0.545 (*F*)	0.909	0.496	0.333	0.373
2. Horned			0.506 (*F*)	0.909	0.500	0.333	0.375
ILSTS017:							
1. Polled	105-125	4	0.500 (*D*)	1.000	0.750	8.100	0.500
2. Horned			0.337 (*I*)	1.000	0.860	6.400	0.720
All loci							
1. Polled		2.800		0.682	0.517	2.672	0.505
2. Horned				0.771	0.506	2.001	0.555

n_A = Number of Alleles
FNA = frequency of the most frequent allele
H_o = observed heterozygosity
H_e = expected heterozygosity
HWE = Hardy Weinberg Equilibrium
PIC = Polymorphic Information Contents

Observed heterozygosity ranged from 0.00 (HEL9) to 1.00 (ILSTS017 and ILSTS045) with a mean of 0.68 (polled) and 0.77 (horned), while expected heterozygosity ranged from 0.00 (HEL9) to 0.86 (ILSTS017) with a mean of 0.51 (polled) and 0.50 (horned). The difference between the observed heterozygosity values and the expected heterozygosity indicates that there has not been a Hardy-Weinberg equilibrium. Changes in gene frequency from one generation to another that may be caused by selection, migration, mutation and genetic drift. In general, the observed heterozygosity is higher than the expected heterozygosity, which may be due to the presence of more heterozygous individuals in the sample analyzed

The PIC value ranged from 0.00 (HEL9) to 1.50 (ILSTS045) with a mean of 0.50 (polled) and 0.55 (horned). The PIC value is low if the PIC value <0.25, which is found in HEL9 and INRA035. Medium PIC values (PIC 0.25 - 0.5) are found on HEL13 and high PIC values (PIC >0.5)

are found in ILSTS045 and ILSTS017. PIC value is one of the parameters that indicates the informative level of a marker. A fairly high PIC value indicates that the sample population is very heterogeneous and that there is little selection for certain characteristics.

Genetic characterization studies are the first step of conservation programs [22]. Especially the genetic structure of native cattle breeds, which contribute to the world animal genetic resources, should be estimated using strong molecular markers [23]. Microsatellite markers are commonly used to estimate both genetic diversity and phylogenetic relationship of domestic cattle breeds of the world.

CONCLUSION

Bali polled cattle have similar genetic characteristics with Bali horns cattle based on HEL9, INRA035, ILSTS045, and HEL13 microsatellites. HEL9, INRA035, ILSTS045, and HEL13 microsatellite can be specifics genetic marker for Bali polled cattle.

ACKNOWLEDGEMENTS

This work was funded by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (RISTEKDIKTI) through the state university innovation Program. Authors wish to thank National Research and Innovation Agency (BRIN) to supporting data analysis.

REFERENCES

[1] W.J.A.Payne and J. Hodges, “Tropical Cattle: Origin, Breeds and Breeding Policies”, Blackwell Science, 1997.

[2] S. Baco, Zulkharnain, R. Malaka, and G. R. Moekti, “Polled bali cattle and potentials for the development of breeding industry in Indonesia”, Hasanuddin J. Anim. Sci., Vol. 2, no. 1, pp. 23–33, 2020.

[3] I. Medugorac, D. Seichter, A. Graf, I. Russ, H. Blum, K. H. Gopel, S. Rothammer, and S. K. Forster, “Bovine polledness – an autosomal dominant trait with allelic heterogeneity”, PloS One,Vol. 7, no. 6, e39477, 2012.

[4] E. J. Cargill, N. J. Nissing, and M. D. Grosz, “Single nucleotide polymorphisms concordant with the horned/polled trait in Holsteins”, BMC Research Notes, Vol. 9, no. 1, pp. 1–9, 2008.

[5] A. Capitan, C.Grohs, B. Weiss, M. N. Rossignol, P. Reversé, and A. Eggen, “A newly described bovine type 2 scurs syndrome segregates with a Frame-Shift mutation in TWIST1”, PLoS One, Vol. 6, no. 7, e22242, 2011.

[6] G. A. Brockmann, J. Martin, T. Friedrich, and S. Manfred, “Marker controlled inheritance of the polled locus in Simmental cattle”, Arch. Tierz., Vol. 43, no. 3, pp.207–212, 2000.

[7] H. Tegelstrom, “Mitochondrial DNA in natural population: An improved routine for screening of genetic variation baed on sensitive silver staining”, Electrophoresis,Vol. 7, pp. 226-229, 1992.
[8] M. D. Bishop, S. M. Kappes, J. W. Keele, R. T. Stone, S. L. F. Sunden, G. A. Hawkinss, S. S. Toldo, R. Fries, M. D. Grosz, J. Yoo, and C. W. Beattle, “A genetic linkage map for cattle”, Genetics,Vol. 136, pp. 619-639, 1994.
[9] D. Vaitman, D. Mercier, K. Moazami–Goudarzi, A. Eggen, R. Ciampolini, A. Lepingle, R. Velmala, J. Kaukinen, S. L. Varvio, P. Martin, and H. Leveziel, “A Set of 99 catlle microsatellites: Characterisation, synteny mapping, and polymorphism”, Mamm. Genome, Vol. 5, no. 5, pp. 288 – 297, 1994.
[10] S. J. Kemp, O. Hishida, J. Wambugu, A. Rink, A. J. Teale, M. L. Longeri, W. Barendse, and A. J. Teale, “A panel of polymorphic bovine, ovine and caprine microsatellite markers. Anim. Genet.,Vol. 26, pp. 299-306, 1995.
[11] E. Armstrong, A. Postiglioni, A. Martinez, G. Rincon, and J. L. Vega-Pla, “Microsatellite analysis of a sample of Uruguayan Creole bulls (*Bos taurus*)”, Genet. Mol. Biol.,Vol. 29, pp. 267-272, 2006.
[12] M. Nei and S. Kumar, “Molecular Evolution and Phylogenetics. Oxford University Press, New York, 2000.
[13] D. L. Hartl and A. G. Clark, “Principle of Population Genetic”, Oxford University Press, USA, 1997.
[14] B. S. Weir, “Genetic Data Analysis: Method for Discrete Population Genetic Data”, Second ed. Sinauer Associates. Sunderland, MA USA, 1996.
[15] M. Metta, S.Kanginakudru, S. Gudisewa, and J. Nagaraju,“Genetic characterization of the Indian cattle breeds, Ongole and Deoni (Bos indicus), using microsatellite markers-a preliminary study”, BMC Genetics, Vol. 5, pp. 1-5, 2004.
[16] A. M. S. Rincon, H.P. Montiel, and G.M.P. Bracamonte, “Assessment of genetic structure in Mexican charolais herds using microsatellite markers”, Mol. Biol. Genet.,Vol. 10, pp. 1-7, 2007.
[17] W. Sun, H. Chen, C. Lei, X. Lei, and Y. Zhang, “Genetic variation in eight Chinese cattle breeds based on the analysis of microsatellite markers”, Genet. Sel. Evol.Vol. 40,pp. 681-692, 2008.
[18] M. V. Chaudhari, S. N. S. Parmar, C. G. Joshi, C. D. Bhong, S. Fatima, M. S. Thakur, and S. S. Thakur, “Moleculer characterization of Kenkatha and Gaolao (*Bos indicus*) cattle breeds using microsatellite markers”, Anim. Biodiv. Conserv., Vol. 32, pp. 71-78, 2009.
[19] Maskur, Muladno, dan B. Tappa, “Identifikasi Genetik Menggunakan Marker Mikrosatelit dan Hubungannya dengan Sifat Kuantitatif pada Sapi”, Media Peternakan, Vol. 30, no. 3, pp. 147–155, 2007.
[20] E. Handiwirawan, R. R. Noor, Muladno, and L. Schuler, “The use of HEL9 and INRA035 microsatellites as specific markers for Bali cattle”, Archiv. fur Tierzucht-Archives of Animal Breeding, Vol. 46, no. 6, pp. 503–512, 2003.

[21] A. K. Pandey, R. Sharma, Y. Singh, B. B. Prakash, and S. P. S. Ahlawat, “Genetic diversity studies of Kherigarh cattle based on microsatellite markers”, J. Genet., Vol. 85, No. 2, pp. 117-122, 2006.
[22] K. S. Devi, B. R. Gupta, and S. Vani, “Genetic diversity and bottleneck studies in Punganur cattle through microsatel- lite markers”, International Journal of Science, Environment and Technology, Vol. 6, no. 1, 303–307, 2017.
[23] E. Demir and M. S. Balcioglu,“Genetic diversity and population structure of four cattle breeds raised in Turkey using microsatellite markers”, Czech. J. Anim. Sci., Vol. 64, pp. 411–419, 2019.
[24] R. R. Noor, Muladno, B. Benyamin, Z. Hedah, and Herliantin, ”The purity test of Bali cattle using protein, DNA microsatellite, hair structure and chromosome analyses”, Faculty of Animal Science IPB and Artificial Insemination Center Singosari, Bogor, 2000.
[25] A. Winaya, “The use of microsatellite markers for detecting polymorphism and phylogeny analyses of cattle genome”, [Thesis] Bogor Agriculture University. Bogor, 2000.
[26] FAO, “Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans Measurement of Domestic Animal Diversity (MoDAD): Recommended Microsatellite Markers, 2004.